

NREM SLEEP HYPERSOMNIA AND REDUCED SLEEP/WAKE CONTINUITY IN A NEUROENDOCRINE MOUSE MODEL OF ANXIETY/DEPRESSION BASED ON CHRONIC CORTICOSTERONE ADMINISTRATION

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Abstract—Sleep/wake disorders are frequently associated with anxiety and depression and to elevated levels of cortisol. Even though these alterations are increasingly sought in animal models, no study has investigated the specific effects of chronic corticosterone (CORT) administration on sleep. We characterized sleep/wake disorders in a neuroendocrine mouse model of anxiety/depression, based on chronic CORT administration in the drinking water (35 µg/ml for 4 weeks, “CORT model”). The CORT model was markedly affected during the dark phase by non-rapid eye movement sleep (NREM) increase without consistent alteration of rapid eye movement (REM) sleep. Total sleep duration (SD) and sleep efficiency (SE) increased concomitantly during both the 24 h and the dark phase, due to the increase in the number of NREM sleep episodes without a change in their mean duration. Conversely, the total duration of wake decreased due to a decrease in the mean duration of wake episodes despite an increase in their number. These results reflect hypersomnia by intrusion of NREM sleep during the active period as well as a decrease in sleep/wake continuity. In addition, NREM sleep was lighter, with an increased electroencephalogram (EEG) theta activity. With regard to REM sleep, the number and the duration of episodes decreased, specifically during the first part of the light period. REM

and NREM sleep changes correlated respectively with the anxiety and the anxiety/depressive-like phenotypes, supporting the notion that studying sleep could be of predictive value for altered emotional behavior. The chronic CORT model in mice that displays hallmark characteristics of anxiety and depression provides an insight into understanding the changes in overall sleep architecture that occur under pathological conditions. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: sleep disorders, hypersomnia, sleep/wake continuity, corticosterone, anxiety/depression, behavior.

INTRODUCTION

Sleep disorders are one of the early clinical symptoms observed in mental diseases, including depression (Riemann et al., 2001). Subjects with altered sleep patterns are ten times more likely to develop depression than individuals without sleep complaints (Taylor et al., 2005). Conversely, 50–90% of depressed patients exhibit poor sleep quality (Armitage, 2007). Sleep disorders encountered in depressive conditions can include some types of insomnia (20–80%) or hypersomnia (10–40%) (Kaplan and Harvey, 2009). According to clinical studies, the main sleep disturbances associated with depression are difficulties to initiate sleep and to maintain it, with early morning awakenings. Moreover, sleep architecture is modified by an increase in rapid eye movement (REM) sleep propensity leading to reduced REM sleep latency, reduced non-rapid eye movement (NREM) sleep and sleep fragmentation. This leads to poor sleep quality and decreased continuity of sleep (Gronli et al., 2004).

Numerous studies have described sleep/wake disorders in stress-related animal models that recapitulate the physiopathology of anxiety/depression but none has focused on the specific effects of chronic corticosterone (CORT) administration on sleep. We recently developed a translational model, based on long-term oral CORT exposure (David et al., 2009), mimicking the hypothalamic–pituitary–adrenal (HPA) axis dysfunctions observed in depressed patients (Nemeroff, 1998; Holsboer, 2000). The CORT model is a chronic exposure method optimized for use in modeling the persistent anxiety/depression-like state in rodents. It allows

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Abbreviations: β-CD, β-cyclodextrin; CORT, corticosterone; CRH, corticotrophin-releasing hormone; EEG, electroencephalogram; EMG, electromyogram; EOG, electrooculogram; GR, glucocorticoid receptor; HPA, hypothalamo–pituitary–adrenal axis; MA(s), microarousal(s); MR, mineralocorticoid receptor; NREM sleep, non-rapid eye movement sleep; NSF, Novelty-suppressed feeding; OF, open field; REM sleep, rapid eye movement sleep; SCN, suprachiasmatic nucleus of the hypothalamus; SE, sleep efficiency (ratio of total sleep time/total recording time); SD, sleep duration; Veh, Vehicle.

the assessment of multiple behavioral tests in the same animals using an etiologically relevant model of depression that is easily replicable between and within laboratories (David et al., 2009; David et al., 2010; Gould, 2011; Mendez-David et al., 2013). Chronic CORT administration induces high emotionality, associated with a decrease in neurogenesis (David et al., 2009) and altered pain sensitivity (Hache et al., 2012). Such behavioral and neurochemical alterations are reversed by classical and innovative antidepressants (Rainer et al., 2011; Hache et al., 2012). Interestingly, our group recently reported in this model a flattened circadian rhythm and decreased activity in the home-cage, especially during the dark phase (Rainer et al., 2011). Because such alterations might parallel sleep/wake modifications, we investigated, in this neuroendocrine mouse model with altered emotional behavior, the actual sleep/wake disorders in relation to the anxi/depressive-like phenotype.

EXPERIMENTAL PROCEDURES

Animals

Twenty-four to twenty six adult male C57BL/6J mice (Janvier Labs, Saint-Quentin Fallavier, France), 7–8 weeks old (20–25 g) at the beginning of the treatment, were used in experiments. Mice were group-housed (five per cage) and kept under standard conditions: 12-h light/dark cycle with lights on at 7:00 AM, 21 °C \pm 1 °C, 60% relative humidity, food (standard A04 SAFE food pellet) and water available *ad libitum*, throughout the experimental period. After a 4-week treatment by corticosterone (CORT-mice) or β -cyclodextrin (β -CD) (vehicle-mice), mice underwent a surgical implantation of electrodes for sleep evaluation. Mice were isolated from the recovery surgical period (1 week) until the end of the experiment in order to prevent the animals gnawing at electrode connectors. Separated groups ($n = 8$ –10) were used to assess novelty-suppressed feeding test to investigate if sleep modifications could be correlated with this composite behavioral test. Throughout the study, we have selected only animals for which we obtained perfect sleep recordings and used behavioral results obtained in the same animals to correlate both parameters (sleep and behavior). All testing was conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee (Council directive 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale; permission 92-256B to D.J.D.).

Drugs and reagents

The dose and duration of vehicle and CORT treatments were selected based on previous studies (David et al., 2009; Rainer et al., 2011). The vehicle solution (0.45% β -CD) was made by dissolving β -CD powder (Sigma–Aldrich, Saint-Quentin Fallavier, France) in water (20%) with magnetic agitation and then diluted until the appropriate concentration (0.45%). The CORT solution (35 μ g/ml equivalent to about 5–7 mg/kg/d), was prepared by

dissolving the CORT powder (Sigma–Aldrich, Saint-Quentin Fallavier, France) by a sonication step in 20% vehicle solution. CORT treated-mice received 35 μ g/ml CORT dissolved in β -CD and control mice receive β -CD (0.45%) alone. All treatments (vehicle and CORT), were administered per os in drinking water available *ad libitum*, delivered in opaque black bottles and changed every 4 days.

Behavioral tests

Open field (OF) test. This behavioral test characterizes anxiety-like behavior (Prut and Belzung, 2003; Dulawa et al., 2004; David et al., 2009). Measured parameters are entries and time spent at the center (defined by 21 \times 21 cm² virtual area and assimilated as an anxiety-like area), at the periphery, locomotor activity and ambulatory distance during 30 min. Infra-red wall sensors in plexiglas open field boxes 43 \times 43 cm² (Med, Associates, Georgia, VT, USA) allowed to record measured parameters. An anxiety-like phenotype was associated with decreased values of number of entries and time spent in the center.

Fur coat state test (CT). This test evaluates the depression-like state of CORT-mice via their grooming capacity by assigning a fur coat state score (Griebel et al., 2002; Santarelli et al., 2003; Surget et al., 2008). The total score of animals was defined as the sum of scores from five body parts. An animal not prone to depression-like phenotype will have a normal grooming activity and a net coat condition with a low score, and conversely.

Novelty-suppressed feeding (NSF). NSF paradigm is a conflict test that elicits competing motivational situation between feeding desire and the aversion that can have animals to move and venture in a novel environment brightly lit at the center (anxiety-like area). As previously described (Santarelli et al., 2003; David et al., 2009), 24 h before the test, animals were put in fasted condition and home cages and grids were changed. NSF apparatus was a rectangular box (50 \times 40 \times 20 cm) filled with sawdust with a similar granule that those usually used and placed at the center of the box. Before the test, each animal was weighed to estimate the loss of weight due to the 24-h food deprivation. This test, carried out over 10 min (maximal period), measured the latency to feed in an aversive environment. After the test, animals were returned to their home cages to evaluate the food consumption of a pellet during 5 min. An anxiety/depressive-like phenotype was linked to increased latency to feed.

All behavioral tests were conducted with and without the knowledge of the treatment arm of the mice studied.

Surgical procedure of implantation of electrodes for polygraphic sleep-wakefulness monitoring

All mice were implanted with electrodes for polygraphic sleep/wake monitoring (enamelled nichrome wire,

diameter 150 μm) on a stereotaxic frame under anesthetic conditions made of a mixture of ketamine (Imalgène 1000)/xylazine (1% Rompun) (100/5 mg/kg, intraperitoneally) (Tissier et al., 1993; Boutrel et al., 1999). Body temperature was maintained at 37 °C using a heating pad and a rectal temperature probe. An application of ophthalmic ointment (Liposic, Chauvin Laboratory, BAUSCH & LOMB, Montpellier, France) was placed on the eyes to prevent drying of the cornea. The skin was previously sanitized with betadine. As previously described (Popa et al., 2005; Real et al., 2007), cortical electroencephalographic (EEG) electrodes were inserted epidurally through the skull over the right cortex (2 mm lateral to the sagittal line, 2 mm posterior to the bregma line) and over the cerebellum (at midline, 2 mm posterior to the lambda line). Electrooculogram (EOG) electrodes were positioned subcutaneously on both sides of the left eye and electromyogram (EMG) electrodes were inserted into the cervical portion of the trapezoid muscles. All polygraphic sleep/wake electrodes were fixed to the skull by acrylic dental cement (Super Bond Dentalon Plus, GACD, France) and then welded to a mini six-channel connector (F206D, Antelec, France) also embedded in cement. After surgery, mice were accustomed to the recording procedure in individualized custom-made Plexiglas recording cages (19 × 19 × 30 cm). Mice were habituated to both the recording wardrobe and custom-made connecting cables (M206D, Antelec, France) during last 4 days of the total recovery period.

Sleep/wake recordings, scoring and sleep parameters

Sleep/wake recordings. Spontaneous sleep/wakefulness recordings were performed during a 24-h period starting at 7:00 PM when the lights went off, in a sound-attenuated, ventilated and temperature-controlled wardrobe. To allow freedom of movement during the recording time, recording cables were connected to a collector rotating ring (ref. SL6C/SB, Bilaney, Plastics One). Polygraphic tracings (EEG, EMG and EOG signals) were amplified and recorded by an Embla A10[®] system and the software Somnologica[®] (Medcare, Reykjavik, Iceland) and fed into a computer at a sampling frequency of 100 Hz for neck EMG and EOG and 200 Hz for EEG (Real et al., 2007). All signals were filtered at 50 Hz to eliminate powerline artifacts.

Scoring. Sleep/wake architecture was analyzed by visual inspection of muscular activity (EMG), eye movements (EOG) and cerebral activity (EEG) signals by scoring 10-s epochs (Somnologica 2 software, Medcare, Reykjavik, Iceland) according to standard criteria (Boutrel et al., 2002; Lena et al., 2004). Thus, waking state was characterized by high muscular activity, eye movements and a cerebral activity with high frequency (8–30 Hz) and low amplitude. NREM sleep was defined by low muscular activity, lack of eye movements and cerebral activity with high amplitude but low frequency (0.5–4.99 Hz). REM sleep was characterized by muscle atonia, bursts of rapid-eye movements and cerebral activity with mixed frequency (5–29.99 Hz) and low amplitude

close to that of wakefulness. Microarousals (MAs) were scored as single events occurring during NREM sleep (Lena et al., 2004). Defined as a transient (less than 10 s) reduction in EEG power, they are frequently associated with several peripheral signs such as a slight muscular (EMG) activation, heart beat acceleration and respiratory events (Lena et al., 2004; Alexandre et al., 2008). Counted as single events that do not cause an increment in the waking time or the number of state transitions, MAs are different from wake episodes. Numbers of MAs were used to assess the stability of NREM sleep (Lena et al., 2004; Alexandre et al., 2008). Because mice have polyphasic sleep, we characterized sleep changes during the whole recording time (24 h) but also distinguished the dark phase (7:00 PM to 7:00 AM) and the light phase (7:00 AM to 7:00 PM).

Sleep/wake parameters. Sleep latencies. NREM sleep latency was visually determined as the duration from at least three consecutive epochs (i.e. 30 s) spent in wakefulness to the appearance of three or more consecutive epochs scored as NREM sleep (Veasey et al., 2000). REM sleep latency was determined by the time elapsed from sleep onset, after the animal had been awakened, to the two first REM sleep epochs (Popa et al., 2005). In this study, sleep latency was measured from the beginning of the dark phase, corresponding to the start of recordings at 7:00 PM after animals have been awakened during 2 min. Since animals were continuously recorded during 24 h and to avoid environmental influences that might have represented confounding factors in sleep/wake recordings (i.e. not to wake up spontaneously sleeping animals), sleep latency in the light phase was measured individually from the moment when animals were awake after lights on.

Total sleep duration (SD) and sleep efficiency (SE). Total sleep duration was investigated summing the time spent in NREM sleep and the time spent in REM sleep, during 24 h, the dark and the light phases (Veasey et al., 2000). Sleep efficiency was obtained by dividing the time spent in both NREM and REM sleep (sleep duration) by the corresponding recording time (24-h, 12-h dark and 12-h light) (Veasey et al., 2000).

State transitions. As previously described (Takahashi et al., 2008), sleep/wake transitions from one vigilance state to another were determined in order to investigate the sleep/wake continuity (Lena et al., 2004; Alexandre et al., 2006). For transitions from NREM sleep to wake, onset of wake was determined by the first sign of EEG desynchronization. Onset of sleep was defined by the first appearance of synchronized EEG. For NREM sleep to REM sleep and REM sleep to wake transitions, the onset and end of REM sleep were defined, respectively, by the appearance of continuous rhythmic theta waves on the cortical EEG and by the interruption of sustained theta waves and the onset of desynchronized EEG.

EEG power spectral analysis. We performed a classical power spectrum analysis (Alexandre et al.,

2008; Popa et al., 2008) using the Somnologica® software (Medcare, Reykjavik, Iceland). For each animal, a spectrogram was obtained by fast Fourier transform (FFT) on 10 s periods and the values for power spectra were divided into four frequency bands: delta (0.5–4.99 Hz), theta (5–9.99 Hz), alpha (10–14.99 Hz) and beta (15–29.99 Hz). The EEG power spectra for each of the four frequency bands were expressed as a percentage of the mean EEG power over all frequency bands and were compared between vehicle and CORT-treated mice.

Study design

The animals were subjected to 4 weeks of phenotypic induction (35 µg/ml in drinking water) or maintained in control condition. Subsequently, the behavioral study was performed including the open field paradigm, the novelty-suppressed feeding test and the fur coat state score test. After validation of the anxio/depressive-like phenotype, animals were submitted to polysomnographic electrodes surgery and then left for a week's recovery before sleep/wake recordings.

Statistical analysis

All results (behavioral tests and sleep/wake records) were expressed as mean \pm standard error mean (S.E.M.). All data were analyzed by Statview 5.0 software (SAS Institute Inc., Cary, NC, USA). Significance was tested according to Student *T*-test or an analysis of variance (two-way ANOVA) using treatment and time as factors and followed by a post-hoc Fischer's PLSD test in the case of statistically significant differences between the treatment groups. Relationships between behavioral parameters and sleep/wake impaired parameters were assessed by Pearson's correlation analysis. The significance level was set at: $*p < 0.05$.

RESULTS

Changes in the sleep/wake architecture

Considering that mice have polyphasic sleep, we investigated the variation of sleep/wake architecture during 24 h, the dark and the light phases separately.

A holistic view of 24-h sleep/wake recordings of each treatment group is indicated by hypnograms in Fig. 3E.

Sleep duration and sleep efficiency. In CORT-treated mice compared with controls, an increase in the total sleep duration (+4.8%, Fig. 1A) and sleep efficiency (+3.7%, Fig. 1D) was observed during the 24-h recording ($F_{1,48} = 5.2$; $*p < 0.05$ and $F_{1,48} = 5.3$; $*p < 0.05$).

These modifications were significant only during the dark phase, with total sleep duration was raised by 10.9% ($F_{1,48} = 6.5$; $*p < 0.05$; Fig. 1B) and sleep efficiency by 9.2% ($F_{1,48} = 6.6$; $*p < 0.05$; Fig. 1E) compared with controls.

In the light phase, total sleep duration (Fig. 1C) and sleep efficiency (Fig. 1F) were not different from controls (479.35 \pm 7.34 min in CORT-treated mice versus 475.08 \pm 5.83 min in controls; $F_{1,48} = 0.2$; $p = 0.65$

and 0.67 \pm 0.001 in CORT-treated mice versus 0.66 \pm 0.008 in controls; $F_{1,48} = 0.2$; $p = 0.65$, respectively).

Duration and number of sleep/wake episodes. During the dark phase. In CORT-treated mice compared with controls, total wake duration decreased (−7.8%) during several hourly periods ($F_{1,48} = 6.5$; $*p < 0.05$; Fig. 2A) to the benefit of a 12.4% increase in total NREM sleep duration ($F_{1,48} = 9.4$; $**p < 0.01$; Fig. 2C). In this same time window, the mean duration of wake episodes decreased by 20.2% (Fig. 2B) while their number increased by 18.6% (Fig. 3A) ($F_{1,48} = 14.5$; $***p < 0.001$ and $F_{1,48} = 4.2$; $*p < 0.05$, respectively). During the dark phase, the number of NREM sleep episodes increased by 19% ($F_{1,48} = 4.3$; $*p < 0.05$; Fig. 3B) while their mean duration was unchanged (4.4 \pm 0.22 min in CORT-treated mice versus 4.53 \pm 0.18 min in controls). Moreover, CORT-treated mice exhibited more microarousal events than controls (+36.4%, $F_{1,48} = 5.7$; $*p < 0.05$; Fig. 3D). Thus, during the dark phase when mice are essentially active, CORT-treated mice have more intrusion of NREM sleep episodes (Figs. 2C, 3B) that interrupts wake episodes, the latter being therefore more numerous but of shorter mean duration (Figs. 2A, B, 3A, E).

During the light phase. In CORT-treated mice compared with controls, the major sleep changes observed during the light phase concerned mainly REM sleep. Thus, there was a 14.8% decrease in the total REM sleep duration from 08:00 AM to 10:00 AM ($F_{1,48} = 12.9$; $***p < 0.001$; Figs. 2E, 3E). A similar trend of decreased number of REM sleep episodes was observed (47.87 \pm 2.16 versus 42.31 \pm 2.03; $F_{1,48} = 3.5$; $p = 0.06$) (Fig. 3C) while their mean duration was unchanged (1.25 \pm 0.37 min in CORT-treated mice versus 1.29 \pm 0.033 min in controls, $F_{1,48} = 1.3$; $p = 0.26$; Fig. 2F).

Transitions between sleep/wake stages. During the dark phase, CORT-treated mice exhibited an increase in the number of transitions from wake to NREM sleep (+16.3%; $F_{1,48} = 4.2$; $*p < 0.05$) and from NREM sleep to wake (+29.1%; $F_{1,48} = 5.8$; $*p < 0.05$) (Fig. 4A). These increases parallel those observed in the number of wake and NREM sleep episodes described above (Fig. 3A, B).

During the light phase, an increase in the number of transitions from NREM sleep to wake (+34.8%; $F_{1,48} = 7.9$; $**p < 0.01$) and a decrease in those from NREM sleep to REM sleep (−18.5%; $F_{1,48} = 13.8$; $***p < 0.001$) and from REM sleep to wake (−18.6%; $F_{1,48} = 13.9$; $***p < 0.001$) was observed in CORT-treated mice compared to controls (Fig. 4B). These modifications parallel the decreased number and total duration of REM sleep episodes observed at the beginning of the light phase in CORT-treated mice as described above (Fig. 2E, Fig. 3C).

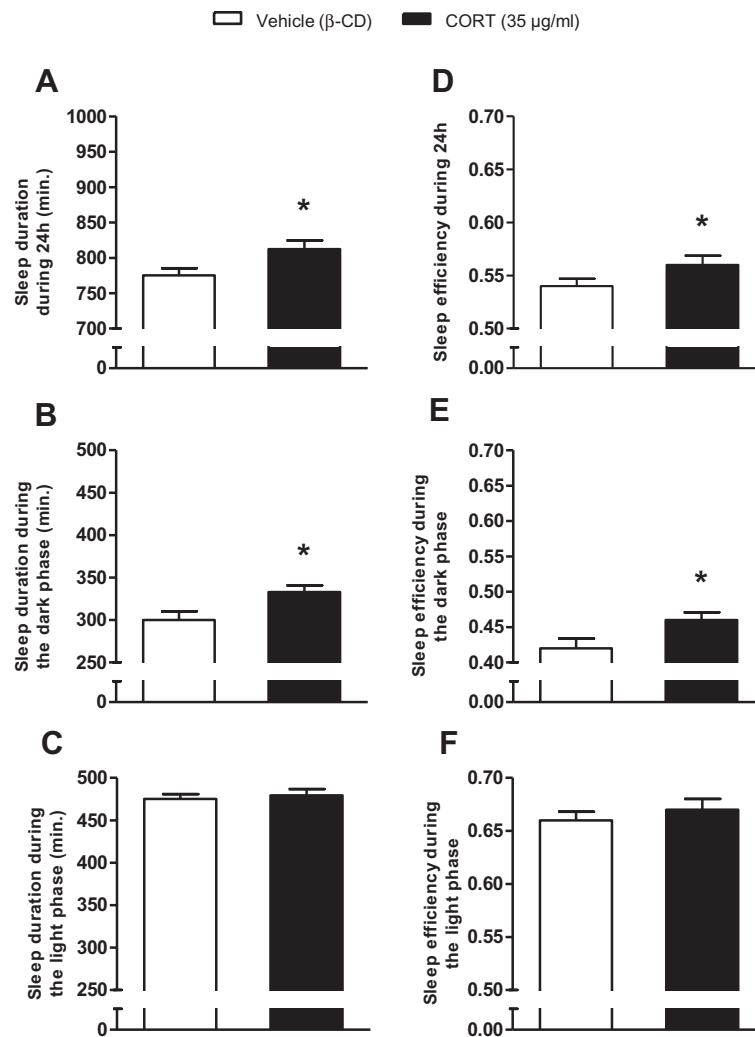


Fig. 1. Effects of a 4-week corticosterone treatment (35 µg/ml per os) on sleep duration and sleep efficiency. Total sleep duration during (A) the 24-h recording time, (B) over the dark phase (12 h) and (C) over the light phase (12 h). Sleep efficiency during (D) the 24-h recording time, (E) over the dark phase (12 h) and (F) over the light phase (12 h). Values plotted are mean \pm S.E.M. ($n = 24$ –26 per groups); * $p < 0.05$ in ANOVA analysis followed by a Fischer's post hoc test in comparison to the β -cyclodextrin (0.45%)-treated group. β -CD, β -cyclodextrin; CORT, corticosterone.

NREM and REM sleep latencies. During the dark phase, CORT-treated mice exhibited no significant change in NREM sleep latency (34 ± 8 min in CORT-treated mice versus 24.9 ± 6.7 min in controls; $F_{1,48} = 0.8$; $p = 0.38$) nor REM sleep latency (82 ± 16 min in CORT-treated mice versus 69.8 ± 15.6 min in controls; $F_{1,48} = 0.3$; $p = 0.6$). Likewise to the dark period, there was no significant changes during the light phase in NREM (28.6 ± 4.4 min in CORT-treated mice versus 25.8 ± 3.8 min in controls; $F_{1,48} = 0.2$; $p = 0.6$) and REM (13.4 ± 1.7 min in CORT-treated mice versus 16.3 ± 2.1 min in controls; $F_{1,48} = 1.2$; $p = 0.3$) sleep latencies.

EEG power spectrum analysis

In CORT-treated animals, the power of the theta band (5–9.99 Hz) during NREM sleep increased by 5.2% during the 24-h recording time ($F_{1,48} = 4.4$; * $p < 0.05$;

Table 1). The other bands (delta, alpha and beta) were not altered.

During the dark phase, after chronic CORT-treatment, a 9.1% increase in the NREM EEG power spectrum for theta waves ($F_{1,46} = 10.5$; ** $p < 0.01$; Table 1) was noted, as during the 24-h recording time, without EEG power spectrum compensation in other analyzed vigilance states.

During the light phase, there were no significant changes in EEG power spectrum regardless of the sleep/wake stage (see Table 1).

Emotion-related behavior in CORT-treated mice

In the open field paradigm, the number of entries in the center decreased by 30.75% ($F_{1,48} = 23.07$; *** $p < 0.001$; Fig. 5A), time in the center by 36.5% ($F_{1,48} = 17.6$; *** $p < 0.001$; Fig. 5B) and distance ratio by 13.3% ($F_{1,48} = 6.4$; * $p < 0.05$; Fig. 5C) in CORT-treated mice compared with controls.

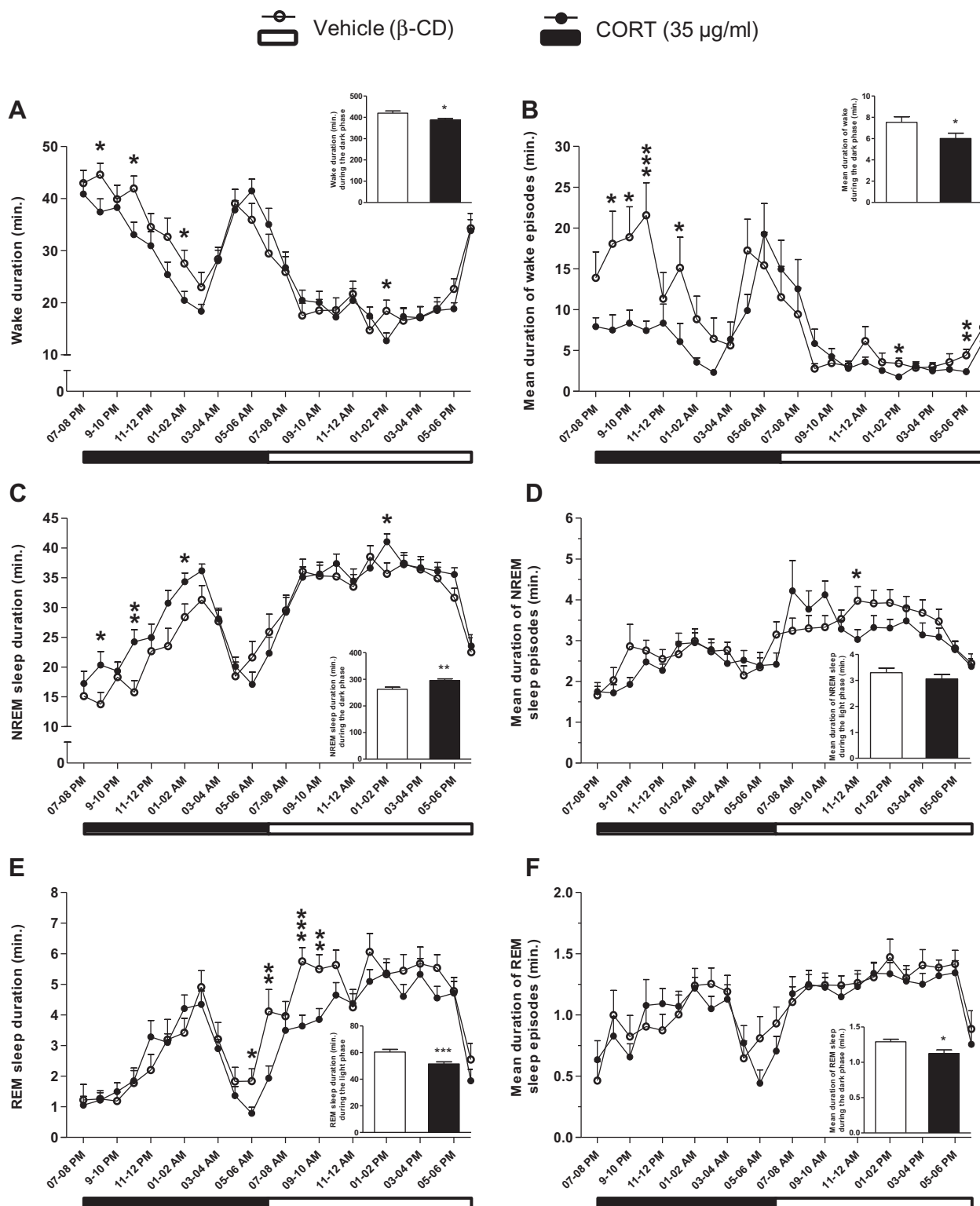


Fig. 2. Effects of a 4-week corticosterone treatment (35 µg/ml per os) on the sleep/wake pattern during the 24-h recording time. (A) Total wake duration (minutes). (B) Mean duration of wake episodes (per hour). (C) Total NREM sleep duration (minutes). (D) Mean duration of NREM sleep (per hour). (E) Total REM sleep duration (minutes). (F) Mean duration of REM sleep (per hour). Values plotted are mean \pm S.E.M. ($n = 24$ –26 per groups); * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ in ANOVA analysis followed by a Fischer's post hoc test in comparison to the β -cyclodextrin (0.45%)-treated group. β -CD, β -cyclodextrin; CORT, corticosterone. Black bars under the X-axis represent the dark phase (7:00 PM to 7:00 AM) and white bars the light phase.

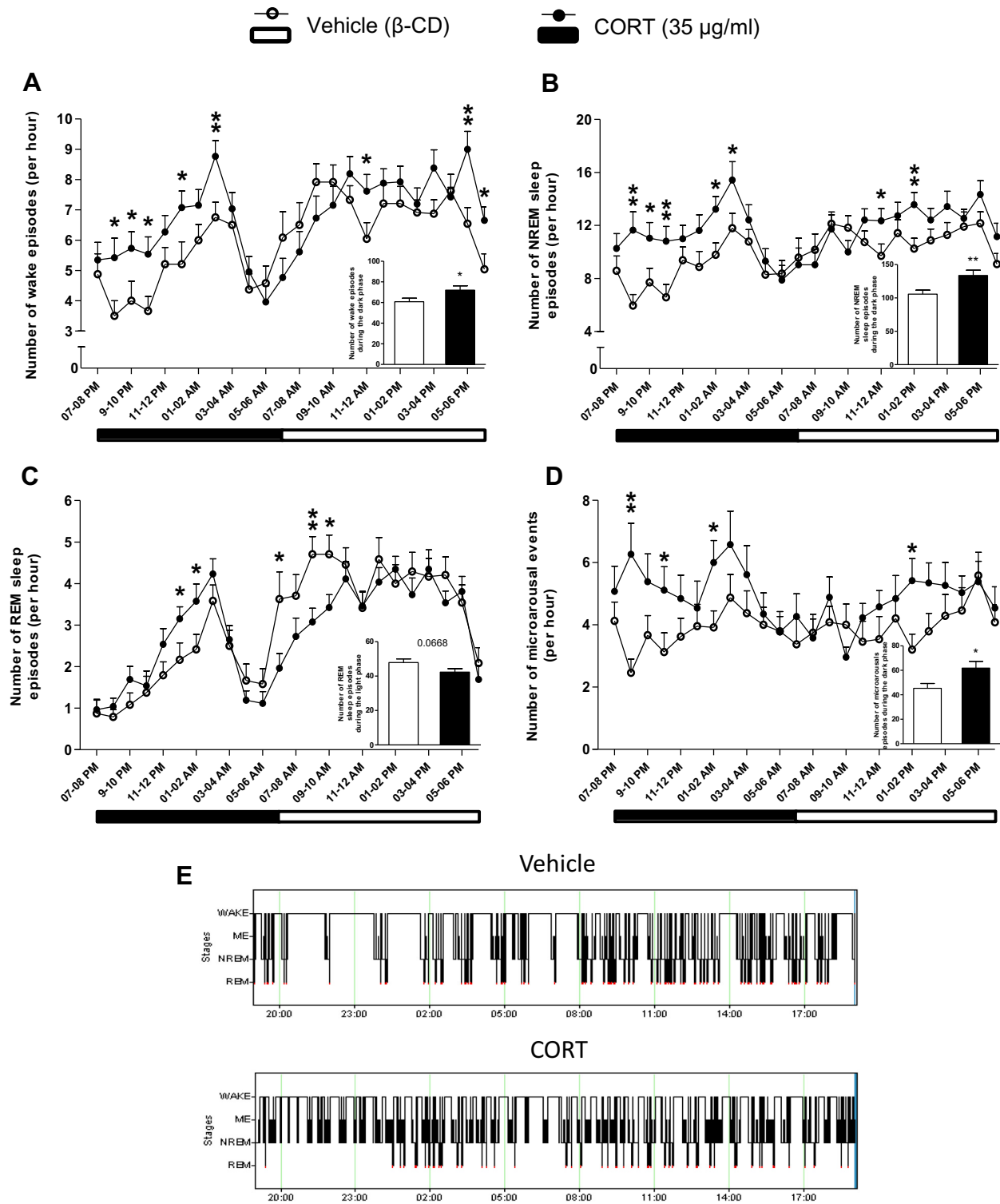


Fig. 3. Effects of a 4-week corticosterone treatment (35 $\mu\text{g/ml}$ per os) on the number of wake (A), NREM sleep (B), and REM sleep (C) episodes and micro-arousal (D) events per hour and during the 24-h recording time. Values plotted are mean \pm S.E.M. ($n = 24\text{--}26$ per groups); * $p < 0.05$; ** $p < 0.01$ in ANOVA analysis followed by a Fischer's post hoc test in comparison to the β -cyclodextrin (0.45%)-treated group. β -CD, β -cyclodextrin; CORT, corticosterone. Black bars under the X-axis represent the dark phase (07:00 PM to 07:00 AM) and white bars the light phase. (E) Representative hypnograms of a vehicle-treated mouse (top) and of a CORT-treated mouse (bottom). ME, microarousal events; NREM, NREM sleep episodes; REM, REM sleep episodes.

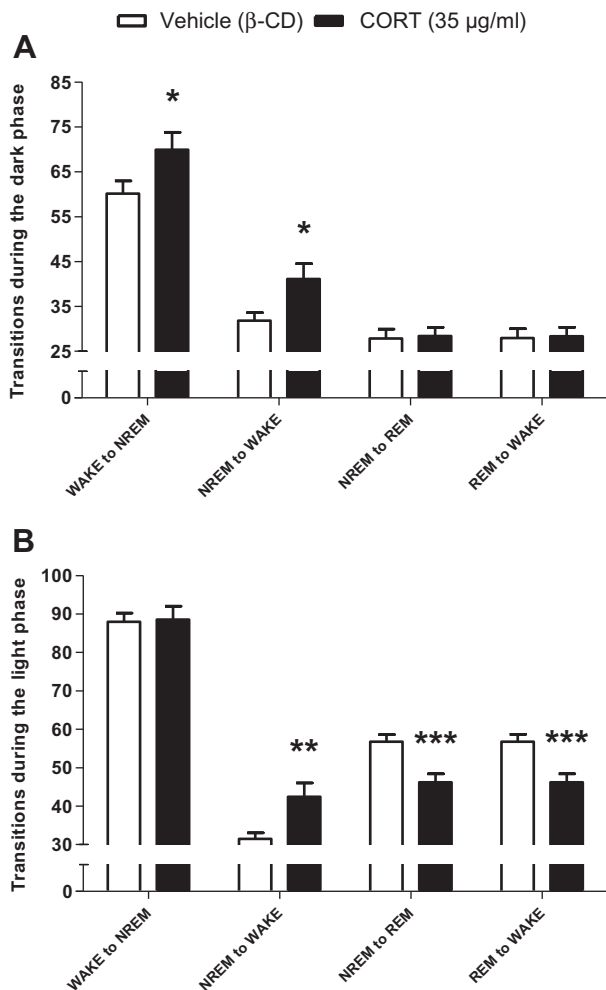


Fig. 4. Effects of a 4-week corticosterone treatment (35 μ g/ml per os) on the sleep/wake transitions. (A) Number of transitions between vigilance states during the dark phase (12 h). (B) Number of transitions between vigilance states during the light phase (12 h). Values plotted are mean \pm S.E.M. ($n = 24$ – 26 per groups); * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ in ANOVA analysis followed by a Fischer's post hoc test in comparison to the β -cyclodextrin (0.45%) treated group. β -CD, β -cyclodextrin; CORT, corticosterone.

In the fur coat state score test, chronic CORT treatment increased the deterioration of fur coat state by 1164.4% ($F_{1,48} = 57.5$; *** $p < 0.001$; Fig. 5D).

In the NSF, CORT-treated mice exhibited an increase by 86.4% in the latency to feed ($F_{1,21} = 14.9$; *** $p < 0.001$; Fig. 5E) without any changes in home-cage food consumption (0.614 ± 0.037 versus 0.595 ± 0.075 , $F_{1,21} = 0.055$; $p = 0.8173$).

Correlations between sleep/wake architecture and behavioral parameters in the CORT model; prediction of the anxiety/depressive-like phenotype by sleep/wake alterations

To determine the relationship between anxio/depressive-like behavior and sleep/wake alterations, we tested correlations between both types of parameters.

In an anxiety-sleep correlation analysis, under CORT-treatment, entries in the center of the open field correlated

with the number of REM sleep episodes during the 24-h recording time ($F_{1,25} = 7.9$; $R^2 = 0.248$; $p = 0.0096$; Fig. 6A) and with the number of REM sleep episodes during the light phase ($F_{1,25} = 4.6$; $R^2 = 0.163$; $p = 0.0411$; Fig. 6B).

In an anxio/depressive-sleep correlation analysis, under CORT condition, the latency to feed in the NSF correlated with the NREM duration during the dark phase ($F_{1,7} = 7.97$; $R^2 = 0.571$; $p = 0.0302$; Fig. 6C). Animals that reached the cut-off of 600 s in the NSF paradigm were excluded for behavioral-sleep correlation analysis in order to avoid any plateau effect in the correlation.

DISCUSSION

Based on the assumption of the existence of disturbed circadian rhythms (Rainer et al., 2011), the present study aimed to investigate and characterize sleep/wake disorders in the CORT-treated mouse model of anxiety/depression.

Chronic CORT administration led to NREM sleep hypersomnia and decreased sleep/wake continuity

Chronic CORT administration induced selective changes in both the structure and continuity of sleep in mice. Total sleep duration increased, due to intrusion of NREM sleep into wakefulness during the active period (dark phase), suggesting NREM sleep hypersomnia. NREM sleep was lighter, closer to the waking state, due to increased theta waves and denoting lower sleep quality. Sleep enhancement reduced the total wake duration while the number of wake episodes and the number of state transitions increased, showing less sleep/wake continuity. Furthermore, REM and NREM sleep disturbances respectively correlated with anxiety (through entries into the center of the OF) and anxiety/depressive-like (through the latency to feed in the NSF) phenotype.

These changes confirmed and extended previous data showing decreased activity in the home-cage during the dark phase (Rainer et al., 2011). Our results are in line with other preclinical studies where decreased activity level (Solberg et al., 1999) and increased NREM sleep were observed in a mouse model of extreme trait anxiety (Jakubcakova et al., 2012) or in chronic mild stress procedure in rats (Gorka et al., 1996). NREM sleep displayed less stability due to MAs and was lighter due to an increase in theta waves during both the 24 h and the dark phase. Theta oscillations (5–9.99 Hz), expressed during both REM sleep and wake, are essential in neural communication across deep brain areas to cortical structures (Lesting et al., 2013). Theta waves are putative markers of cortical activation and correlate with limbic activity (Buzsaki, 2002; Rutishauser et al., 2010). Interestingly, theta waves have been implicated in stress-related disorders including anxiety, schizophrenia and autism (Sohal, 2012). Indeed, in mice, theta waves have been linked to anxiety in the open field and elevated plus maze paradigms (Gordon et al., 2005; Adhikari et al., 2010).

Table 1. Effects of a 4-week corticosterone treatment (35 µg/ml per os) on EEG power spectrum during wake, NREM sleep and REM sleep in vehicle and CORT-treated animals

Period	% Total power	WAKE		NREM sleep		REM sleep	
		Veh	CORT	Veh	CORT	Veh	CORT
24 h	Delta (0.5–4.99 Hz)	34.4 ± 2	35 ± 2	43.9 ± 2.1	44.9 ± 2	25.4 ± 1.7	25.7 ± 1.9
	Theta (5–9.99 Hz)	41 ± 0.8	41.8 ± 0.8	32.8 ± 0.6	34.5 ± 0.6*	46.6 ± 1	47.9 ± 1
	Alpha (10–14.99 Hz)	11.5 ± 0.7	11.3 ± 0.6	13.2 ± 0.8	11.6 ± 0.7	13 ± 0.5	13 ± 0.6
	Beta (15–29.99 Hz)	12.8 ± 1.6	11.3 ± 1.1	10.1 ± 1.4	8.9 ± 1	14.7 ± 1.4	12.8 ± 0.9
Dark phase (07:00 PM–07:00 AM)	Delta (0.5–4.99 Hz)	32.5 ± 1.9	33 ± 2	45.5 ± 2.1	44.3 ± 2	26.3 ± 1.9	25.5 ± 1.8
	Theta (5–9.99 Hz)	42.4 ± 0.8	43 ± 0.8	32 ± 0.6	34.9 ± 0.6**	46.1 ± 1	48.1 ± 0.9
	Alpha (10–14.99 Hz)	11.9 ± 0.7	11.8 ± 0.7	12.4 ± 0.8	11.5 ± 0.7	12.7 ± 0.6	12.9 ± 0.7
	Beta (15–29.99 Hz)	12.9 ± 1.6	11.6 ± 1.1	10 ± 1.4	9.3 ± 1.1	14.5 ± 1.4	12.9 ± 1
Light phase (07:00 AM–07:00 PM)	Delta (0.5–4.99 Hz)	37.8 ± 2.1	38.6 ± 2	42.9 ± 2	45.2 ± 2.1	25.1 ± 1.7	26.2 ± 2.3
	Theta (5–9.99 Hz)	38.6 ± 0.8	39.8 ± 0.7	33.2 ± 0.6	34.1 ± 0.7	46.7 ± 1	47.5 ± 1.3
	Alpha (10–14.99 Hz)	10.9 ± 0.7	10.5 ± 0.6	13.6 ± 0.8	11.7 ± 0.8	13.1 ± 0.5	13 ± 0.6
	Beta (15–29.99 Hz)	12.5 ± 1.5	10.8 ± 1	10.2 ± 1.4	8.8 ± 1	14.7 ± 1.4	12.7 ± 0.9

Veh, vehicle; CORT, corticosterone; NREM sleep, non-rapid eye movement sleep; REM sleep, rapid eye movement sleep.

* $p < 0.05$.

** $p < 0.01$.

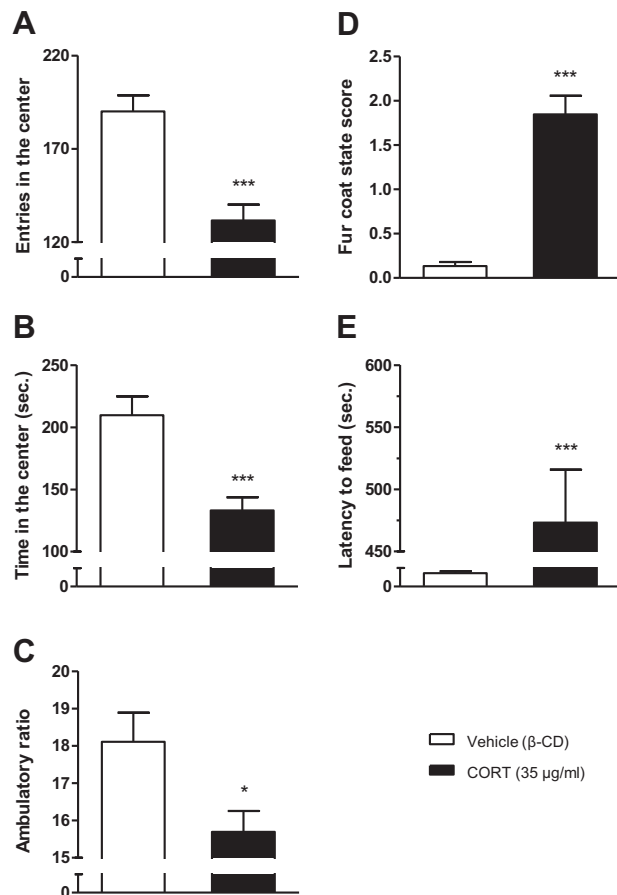


Fig. 5. Effects of a 4-week corticosterone treatment (35 µg/ml per os) in behavioral paradigms. (A) Entries in the center (seconds). (B) Time in the center (seconds). (C) Ambulatory ratio (%) (distance in the center of the open field/total ambulatory distance). (D) Fur coat state score. (E) Latency to feed (seconds) in the novelty-suppressed feeding test. Values plotted are mean ± S.E.M. ($n = 24$ –26 per groups in the open field and coat test and $n = 8$ –10 in the novelty-suppressed feeding test); * $p < 0.05$ and *** $p < 0.001$ in ANOVA analysis followed by a Fischer's post hoc test in comparison to the β-cyclodextrin (0.45%)-treated group. β-CD, β-cyclodextrin; CORT, corticosterone.

However, highly stress-reactive (HR) mice exhibit an enhanced theta activity parallel to an increase in wake and REM sleep duration (Fenzl et al., 2011). Thus, anxiety-like behavior seems to be related to modifications in theta oscillations exchanged and synchronized between the hippocampus, amygdala and prefrontal cortex (Sohal, 2012). In agreement with other stress-related animal models (Tiba et al., 2004; Jha et al., 2005; Pawlyk et al., 2005; Sanford et al., 2010; Philbert et al., 2011; Reyes Prieto et al., 2012), we found a REM sleep inhibition, due to a decrease in the number of REM sleep episodes, especially during the beginning of the light phase. However, our observations contrast with some preclinical models in which REM sleep disinhibition was usually found (Dugovic et al., 1999; El Yacoubi et al., 2011; Fenzl et al., 2011; Jakubcakova et al., 2012; Mrdalj et al., 2013). Finally, we did not observe a significant change in REM sleep latency in the CORT-treated model, in agreement with results obtained in rats after four weeks of mild stress (Gronli et al., 2004) or after five weeks where REM sleep latency was only transiently reduced (Cheeta et al., 1997). These sleep/wake differences between animal models seem to be emotionality-dependent, whether animals are anxious and/or depressed, thus reflecting different mechanisms of pathogenesis of sleep disorders.

With regard to humans, our results were somewhat unexpected. Indeed, up to 80% of depressed patients suffer from insomnia with increased REM sleep pressure and decreased delta sleep whereas only 15–20% of them exhibit hypersomnia (Riemann et al., 2001; Kaplan and Harvey, 2009). However, our observations are in line with the frequent awakenings and lower sleep continuity often reported in depressed patients (Sharpley and Cowen, 1995) and might also be related to the anxiety profile (Fuller et al., 1997). Interestingly, sleep/wake disruptions observed here are close to a subpopulation of depressed patients. These atypically depressed patients exhibit a decrease in HPA axis activity, corticotrophin-releasing hormone (CRH) secretion and hypoactivity of the waking systems thus leading to

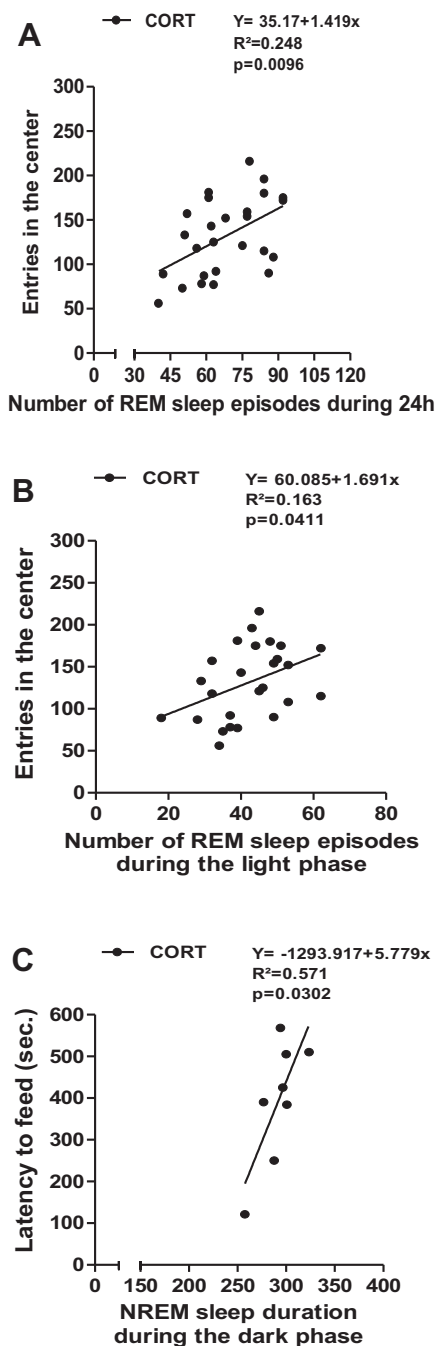


Fig. 6. Correlations between anxiety-like behavior and sleep disorders. (A) Entries in the center (OF) = $f(\text{number of REM sleep episodes during 24 h})$, $R^2_{\text{CORT}} = 0.248$ and $p = 0.0096$; (B) Entries in the center (OF) = $f(\text{number of REM sleep episodes during the light phase})$, $R^2_{\text{CORT}} = 0.163$ and $p = 0.0411$; (C) Latency to feed (NSF) = $f(\text{total NREM sleep duration during the dark phase})$, $R^2_{\text{CORT}} = 0.571$ and $p = 0.0302$. CORT-treated mice are represented in black dots. CORT, corticosterone.

hypersomnia; while REM sleep may not be altered (Antonijevic, 2008).

Several mechanisms may be responsible for sleep/wake impairments observed in the CORT-treated mice. Low doses of glucocorticoids had different effects on sleep, depending on the corticoid receptor subtype (Born et al., 1991; Trapp et al., 1994). For instance,

glucocorticoids increase NREM sleep through a mineralocorticoid receptor (MR)-dependant mechanism and decrease REM sleep through a glucocorticoid receptor (GR)-dependant mechanism (Fehm et al., 1986; Born et al., 1989). These receptors are known to control molecular clock gene expression showing positive (GRE) or negative (nGRE) glucocorticoid-responsive elements (So et al., 2009; Surjit et al., 2011). However, as neither GR nor MR expression has been detected in the suprachiasmatic nucleus of the hypothalamus (SCN) (Ahima and Harlan, 1990; Ahima et al., 1991), CORT effects on sleep/wake architecture, might be indirectly transmitted to the SCN via projections of neuronal populations in other brain areas (Jacobson, 2005; Kolber et al., 2008; Berardelli et al., 2013) expressing GR and/or MR (Dickmeis et al., 2013). Another explanation would be the involvement of CRH receptors which have been shown to decrease REM sleep through a CRH receptor type 1-dependent-mechanism (Romanowski et al., 2010), whereas CRH receptors type 2 would increase it (Liu et al., 2009). Chronic CORT may change HPA axis activity and alter neurohormonal secretion, such as the CRH, which is known to influence sleep (Steiger, 2002). As previously discussed (David et al., 2009), the main mechanisms underlying sleep and behavioral alteration are probably a consequence of chronic CORT-treatment that triggers a negative feedback control on the HPA axis. The fact that sleep modifications occurred more during the dark period are unlikely to be a consequence of a change in CORT levels only during the times when the mice are drinking, but rather result from a chronic impairment in the HPA axis.

Could sleep/wake disorders be predictive to emotion-related behavior?

Since human sleep disorders are often precursors to depressive episodes, a more detailed analysis of the correlation between sleep and behavioral characteristics might strengthen the relevancy of our model. Given this apparent parallel between human and rodent behavioral impairments, we showed that REM sleep and NREM sleep disturbances respectively correlated with the anxiety-like (OF) and anxiety/depressive-like (NSF) phenotype. Thus, a higher anxiety-like phenotype was correlated with a lower number of REM sleep episodes and a higher anxio/depressive-like phenotype was correlated with a higher NREM sleep duration. Conversely to other animal models, the increase of the depression score was related to an increase in the mean duration and the number of REM sleep episodes and to a decrease in the time spent in slow wave sleep (Gronli et al., 2004; Popa et al., 2008). We started to investigate the timing of sleep/wake disorders pathogenesis and their relationship with behavioral dysfunctions. Preliminary results indicate that these disorders are present as early as two weeks of CORT-treatment.

CONCLUSION

Prolonged CORT administration predisposes the present mouse model to changes in sleep/wake architecture.

NREM sleep hypersomnia and decrease in sleep/wake continuity emphasize a unique feature of the CORT model. Its relevancy makes it useful for approaching underlying mechanisms, as it provides a preclinical model for sleep/wake studies associated with mood disorders. This model displaying hallmark characteristics of anxiety and depression provides a novel insight into understanding the changes in overall sleep architecture that occur under pathological conditions, and may be tested during reversal by antidepressant therapies. Thus, this animal model might represent a useful tool for characterizing innovative treatment for sleep/wake disorders with co-morbid anxiety/depression.

COMPETING INTERESTS

Denis David and Jean-Philippe Guilloux currently receive investigator-initiated research support from Lundbeck and served as consultants in the areas of target identification and validation and new compound development for Lundbeck, Roche and Servier in 2011–13.

Bruno Guiard currently receives investigator-initiated research support from Neurosearch and served as a consultant in the areas of target identification and validation and new compound development for Lundbeck and Servier in 2011–13.

AUTHORS' CONTRIBUTIONS

YLD, DJD and PE designed the study.

YLD and GH conducted experiments.

YLD, GH, JPG, BPG, DJD, JA and PE performed data analysis.

YLD, JPG, BPG, DJD, JA and PE wrote or contributed to the writing of the manuscript.

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